Aminoacidurias: Clinical and molecular aspects

SMR Camargo[1], D Bockenhauer[2] and R Kleta[3]

[1]*Institute of Physiology and Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland;* [2]*London Epithelial Group, Great Ormond Street Hospital for Children NHS Trust, London, UK and* [3]*London Epithelial Group, Centre for Nephrology, University College London, London, UK*

Inherited aminoacidurias are caused by defective amino-acid transport through renal (reabsorption) and in many cases also small intestinal epithelia (absorption). Recently, many of the genes causing this abnormal transport have been molecularly identified. In this review, we summarize the latest findings in the clinical and molecular aspects concerning the principal aminoacidurias, cystinuria, lysinuric protein intolerance, Hartnup disorder, iminoglycinuria, and dicarboxylic aminoaciduria. Signs, symptoms, diagnosis, treatment, causative or candidate genes, functional characterization of the encoded transporters, and animal models are discussed.

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Correspondence: *R Kleta, Centre for Nephrology, University College London, Royal Free Hospital/Medical School, Rowland Hill Street, London NW3 2PF, UK. E-mail: r.kleta@ucl.ac.uk*

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Amino-acid transport is vital to life and for many aspects of physiology and pathophysiology. The study of amino-acid transport in epithelial cells, of intestinal or renal proximal tubular origin, and the expression of transporter proteins in heterologous systems, for example, *Xenopus laevis* oocytes, have been immensely fruitful and provided many clues in the past few years. Furthermore, genetic studies in patients with specific aminoacidurias, and phenotypic observations in knockout mice, have brought several major contributions to our understanding of epithelial amino-acid transport. The recognition of changes in individual amino-acid levels in urine and plasma of patients has further supported research and progress in this field.

The association of a disease with an amino-acid transport defect was suggested already a century ago by Sir Archibald Garrod,[1,2] the 'father' of metabolic medicine and biochemical genetics. He described cystinuria as cause of nephrolithiasis in his third and fourth Croonian lectures. In the 1950s, a disorder with pellagra-like symptoms associated with 'constant renal aminoaciduria' was named after the affected members of a British family as Hartnup disorder.[3] Another new rare disorder was first described shortly thereafter in Finnish patients, who excreted large amounts of dibasic amino acids in the urine, had low plasma concentrations of these amino acids, and severe symptoms including coma. The disorder was called lysinuric protein intolerance (LPI) and was later attributed to an epithelial amino-acid transport defect, even though it resembled much more a classical metabolic disorder with signs and symptoms of elevated blood ammonia.[4]

Standard urinary amino-acid screening resulted in the identification of two other asymptomatic aminoacidurias, iminoglycinuria[5–7] and dicarboxylic aminoaciduria.[8,9]

In principal, clinical consequences in specific aminoacidurias can arise from either the deficiency of particular amino acids (lack of absorption in the intestine and urinary loss) or the precipitation of certain amino acids (cystine) in the urine.

The study of aminoacidurias was greatly facilitated by the advent of paper chromatography[10] and then ion exchange chromatography in the 1950s.[11] The latter method can reliably detect and quantify minute amounts of individual amino acids in body fluids like plasma and urine, or even in subcellular compartments. The individual amino acids are separated based on their distinct physicochemical properties

by high-pressure liquid chromatography in specialized columns (packed with cation exchange resins). The principle of the most commonly used assay is the formation of colored compounds from the reaction of amino or imino acids with ninhydrin. The amount of light absorbed at two wavelengths, 570 nm (amino acids) and 440 nm (imino acids), is proportional to the quantity of amino acids present. Even though amino acids can nowadays be detected by different and even more sophisticated methods, ion exchange chromatography has remained the method of choice for clinical practice.

The pathophysiology of aminoacidurias teaches us a lot about epithelial transport physiology. In intestinal and proximal kidney tubule epithelia, specific amino-acid transporters, located on both the luminal and basolateral membranes, together perform an 'uphill' transcellular transport, that is, against the concentration gradient back into the extracellular blood compartment. This net transport is very efficient, and in the renal proximal tubule up to 99% of free amino acids, filtered through the glomerulus, are reabsorbed and thus retained by the organism.

Initially, amino-acid transport types were classified according to specificity and sodium dependence described as systems, a classification that is still used by physiologists. Examples of such systems are, for instance, B^0 (broad selectivity sodium-dependent neutral amino-acid transport), $b^{0,+}$ (broad selectivity sodium-independent neutral and basic amino-acid transport), y^+L (sodium-independent basic amino-acid transport), and X_{AG} (sodium-dependent acidic amino-acid transport).[12] With the increasing number of molecularly identified transporters, a more homogeneous nomenclature was necessary. The solute carrier families (SLC) nomenclature, based on gene homology, was introduced by the Human Genome Organization (HUGO). Unfortunately, some confusion persists, as this system is based on homology rather than on function. In addition, protein names are not necessarily following specific rules. For the purpose of this review, the specific aminoacidurias identified so far (see Table 1) were classified by the chemical properties of the amino acids abnormally excreted (that is, neutral, basic (cationic), acidic (anionic), or imino acids). Amino acids mentioned are L-forms unless specified otherwise. Gene names according to HUGO are mentioned first followed by agreed and commonly used protein names, for example, *SLC6A19* (B^0AT1).

CYSTINURIA (OMIM #220100)

Clinical findings

Patients with cystinuria often present with nephro- or urolithiasis at almost at any age with a clear preference in childhood due to elevated urinary cystine. Kidney stones are radio opaque, although less than calcium-containing ones, and easily diagnosed by ultrasound examinations. Stones often form in the bladder and the presence of a bladder stone in a child should always prompt consideration of cystinuria. Early diagnosis is important, as it allows prevention or diminution of kidney stones. Diagnostically, urinary levels of dibasic amino acids lysine, arginine, and ornithine, and most prominently, of cystine are constantly elevated (for example, cystine up to 50 times normal). Plasma levels of these amino acids in general are at the lower end of the normal range. The clinical problems arise only from the elevated urinary cystine, which precipitates within the urinary tract and forms cystine stones due its low solubility. Urine microscopy reveals

Table 1 | Aminoacidurias genetically elucidated in human

Aminoaciduria	Gene	HUGO	Protein	Chromosome	Hallmark (elevation of individual AA in urine)
Cystinuria A	*SLC3A1*	Solute carrier family 3 (cystine, dibasic, and neutral amino-acid transporters, activator of cystine, dibasic, and neutral amino-acid transport), member 1	rBAT	2p21	Cystine, lysine, arginine, ornithine
Cystinuria B	*SLC7A9*	Solute carrier family 7 (cationic amino-acid transporter, y^+ system), member 9	$b^{0,+}$AT	19q13.11	Cystine, lysine, arginine, ornithine
Cystinuria AB	*SLC3A1*/*SLC7A9*				Cystine, lysine, arginine, ornithine
Lysinuric protein intolerance	*SLC7A7*	Solute carrier family 7 (cationic amino-acid transporter, y^+ system), member 7	y^+LAT1	14q11.2	Lysine, arginine, ornithine
Hartnup disorder	*SLC6A19*	Solute carrier family 6 (neutral amino-acid transporter), member 19	B^0AT1	5p15.33	Neutral amino acids
Iminoglycinuria	?		?	?	Proline, hydroxyproline, glycine
Dicarboxylic aminoaciduria	?		?	?	Aspartate, glutamate

HUGO, Human Genome Organization; NCBI, National Center for Biotechnology Information.
According to HUGO (http://www.genenames.org) and NCBI build 36.2.
? signifies unknown.

characteristic and pathognomonic hexagonal crystals. The cyanide–nitroprusside urinary test is also used, but it is not considered specific.

Treatment

Treatment overall is nonspecific and consists mostly of a high fluid intake to keep the urinary cystine below the solubility threshold of about 1000 μmol l^{-1} (at pH < 7). As cystine solubility increases with pH, alkalization of urine, typically with potassium citrate, can be used. Chelation with D-penicillamine or mercaptopropionylglycine, although highly effective, is rarely used owing to serious side effects. Overall, treatment is cumbersome and some patients lose their kidney function at some time related to recurrent nephro- and urolithiasis.

Physiology and molecular genetics

The overall incidence of cystinuria is estimated to be in the range of 1:7000 births. The consequences of this disease (bladder cystine stones) were first described almost two centuries ago,[13] but the proteins responsible for the disorder were only molecularly defined in the last two decades. Importantly, Charles Dent[14] recognized that not only urinary cystine but also lysine and arginine were constantly elevated in cystinuria patients. Minor urinary elevations of ornithine were appreciated later.

SLC3A1 (rBAT) was the first protein related to epithelial amino-acid transport to be molecularly identified.[15] When expressed in *Xenopus laevis* oocytes, it induced a transport activity for dibasic amino acids and cystine, which was attributed to the presence of an endogenous expressed interacting protein. This interacting protein was later identified as *SLC7A9* ($b^{0,+}$AT).[16,17]

SLC3A1 (rBAT) and *SLC7A9* ($b^{0,+}$AT) are members of the heterodimeric amino-acid transporters family (HAT)[18] mediating dibasic amino acid and cystine transport from the luminal compartment into the cells. The HAT members are composed of two different subunits bound covalently by a disulfide bridge:[19,20] a type II glycoprotein or heavy subunit (in this case *SLC3A1* (rBAT)) and a light subunit (in this case *SLC7A9* ($b^{0,+}$AT)) conferring specificity and being the functional subunit with 12 putative transmembrane domains. Both, which are primarily expressed in kidney and intestine, are localized to the brush border membranes of proximal tubules or enterocytes.[20,21] By overexpression of *SLC3A1* (rBAT) and *SLC7A9* ($b^{0,+}$AT) in an epithelial cell model (Madin–Darby canine kidney, MDCK cells), both proteins were shown to colocalize in the apical membrane, whereas without expression of the heavy subunit *SLC3A1* (rBAT), the light subunit *SLC7A9* ($b^{0,+}$AT) was retained intracellularly.[22] Therefore, *SLC3A1* (rBAT) is essential for proper cell surface expression of *SLC7A9* ($b^{0,+}$AT).[23] The heterodimeric couple exchanges dibasic amino acids and cystine from the tubular lumen for intracellular neutral amino acids[24] (see Figure 1).

Mutations in either interacting subunit *SLC3A1* (rBAT) or *SLC7A9* ($b^{0,+}$AT) cause cystinuria.[15,25] Cystinuria due to mutations in *SLC3A1* (chromosomal locus 2p21) is an autosomal-recessive trait with the heterozygous parents being unaffected, whereas mutations in *SLC7A9* (chromosomal locus 19q13.11), leads to a mild to moderately abnormal urinary amino-acid pattern in most obligate heterozygotes (for example, parents), thus, can be seen as an autosomal-dominant trait. The initial nomenclature, based on the excretion status of obligate heterozygotes (cystinuria types I, II (non-I), III (non-I)), has been 'replaced' by a more meaningful one linked to the genotype.[26] Mutations in *SLC3A1* cause cystinuria type A, mutations in *SLC7A9* cause cystinuria type B, and mutations in both genes (compound heterozygotes) cause cystinuria type AB. The disease severity apparently is the same in cystinuria types A and B.

A mouse model, developed by targeted disruption of the *Slc7a9* gene, exhibited a lithiasic phenotype resembling the classic type B cystinuria. Animals develop massive hyperexcretion of cystine and dibasic amino acids, and cystine crystalluria, with 40% of the animals having cystine calculi in the urinary system.[27] As in humans, oral treatment of this mouse model with D-penicillamine reduced the size and number of calculi.[28]

LYSINURIC PROTEIN INTOLERANCE (OMIM #222700)

Clinical findings

Patients affected by this disorder, in general, come to medical attention from early on in life with several significant problems including failure to thrive and intellectual impairment. Episodes of diarrhea and hyperammonemia, coinciding with increased protein intake (hence the name), can point toward the correct diagnosis. Apparently, hyperammonemia is caused by an interruption of the urea cycle owing to lack of the intermediates required by the cycle (for example, ornithine). These patients, unfortunately, also develop interstitial pneumonia in form of an alveolar proteinosis, hepatomegaly and liver cirrhosis, osteoporosis, and bone marrow involvement. Renal insufficiency can develop due to glomerulonephritis of suspected immunological etiology. Why this specific aminoaciduria presents as a multisystemic disorder with hallmarks of an urea cycle defect and immunological involvement is still a matter of debate.[29]

Elevations of urinary dibasic amino acids, lysine, arginine, and ornithine are diagnostic and more pronounced than in cystinuria; urinary cystine levels in contrast to cystinuria are almost normal (elevated only up to about 2–3 times the norm). In contrast to cystinuria, plasma levels of lysine, arginine, and ornithine tend to be below the norm. Orotic acid and homocitrulline are also elevated and can be of diagnostic value for the discrimination to other urea cycle defects.

Treatment

Treatment is symptomatic and consists of protein restriction, as in other urea cycle disorders, and in this case in supplementation of citrulline. Citrulline is a precursor to both arginine and ornithine, thus can partially restore the defective functions of the urea cycle.

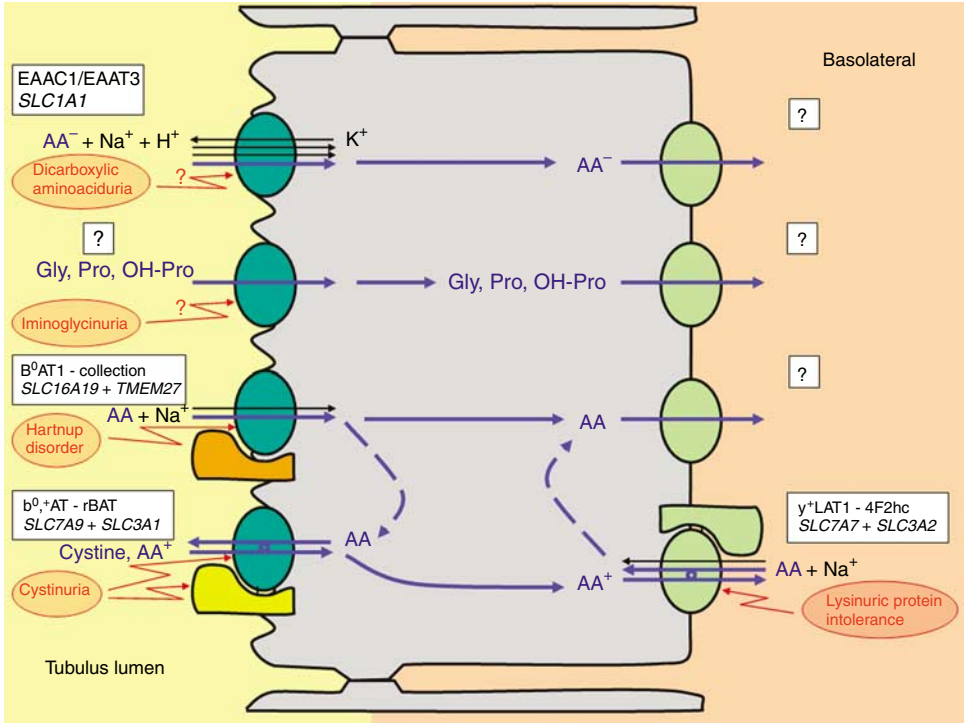

Figure 1 | Simplified scheme of identified amino transport genes/proteins related to amino-acid reabsorption (epithelial transport) in the luminal and basolateral membranes of the renal proximal tubule. So far, only cystinuria, lysinuric protein intolerance, and Hartnup disorder have been elucidated genetically in man. A knock out of *Slc1a1* (Eaac1/Eaat3) causes dicarboxylic aminoaciduria in the mouse. Note that amino-acid transport proteins can have subunits, which are critical for function (heteromeric amino-acid transporters/glycoprotein-associated amino-acid transporters); *Tmem27*'s role for *Slc6a19* was demonstrated in mice. AA, neutral amino acids; AA^+, basic (cationic) amino acids; AA^-, acidic (anionic) amino acids; Gly, glycine; Pro, proline; OH-Pro, hydroxyproline; solid lines indicate directed transport, dashed lines indicate possible recycling processes. Modified from ref. Verrey *et al.*[68]

Physiology and molecular genetics

The incidence of LPI is very low, but reaches in some populations up to 1:50 000 births. It is caused by mutations in *SLC7A7*, which, similar to cystinuria, is a member of the HAT family. The mutations causing this autosomal-recessive disorder have, in contrast to cystinuria, been identified exclusively in the light subunit *SLC7A7* (chromosomal locus 14q11.2)[30,31] mediating transport of dibasic amino acids from intracellular to the basolateral compartment.

Xenopus laevis oocytes expression of its heavy subunit, *SLC3A2* (4F2*hc*), induced transport of neutral and cationic amino acids, but not of cystine, resembling the amino-acid exchange system called y^+L.[32–34] It was later shown that this transport was not mediated by this exogenous heavy chain, but due to an endogenously expressed *Xenopus* light subunit identified as *SLC7A7* (y^+LAT1).[35–37]

SLC3A2 (4F2*hc*) is ubiquitously expressed, whereas *SLC7A7* (y^+LAT1) is highly expressed in intestine, kidney, lungs, and leukocytes.[21,36,37] Similarly to the cystinuria transporter, *SLC7A7*/*SLC3A2* (y^+LAT1/4F2*hc*) localizes to proximal tubules (S1 > S2 ≫ S3) and enterocytes (jejunal and ileal), but in contrast to cystinuria to the basolateral membranes.[21,38] *SLC3A2* (4F2*hc*), the heavy subunit of the basolateral basic amino-acid transporter, functions as a chaperone, assisting in the sorting of the light subunit *SLC7A7* (y^+LAT1) to the plasma membrane.[36,39] When overexpressed in an epithelial cell model (MDCK cells), in the absence of *SLC3A2* (4F2*hc*), only a small fraction of *SLC7A7* (y^+LAT1) appeared at the basolateral surface (putatively associated with endogenous *SLC3A2* (4F2*hc*)). Whereas overexpression of both the heavy and light subunits resulted in their colocalization in the basolateral membrane.[38] This heterodimeric transporter effluxes, with high affinity (micromolar range), dibasic amino acids in exchange for neutral amino acids and Na^+[36] resulting together with the action of $b^{0,+}$AT (SLC7A9) and its subunit rBAT (SLC3A1) in the net reabsorption of dibasic amino acids (see Figure 1).

The significance of the clinical picture in LPI can be explained by a more severe loss of dibasic amino acids in comparison to cystinuria leading to an inefficient urea cycle resulting in hyperammonemia. Obviously, a defect in the basolateral export of dibasic amino acids (as in LPI) has more severe consequences than a defect in the luminal membrane (as in cystinuria). This is, in particular, attributed to the fact that a large fraction of the nutritional amino acids, inclusively dibasic amino acids, are (re)absorbed as di- and tripeptides, which are then hydrolyzed intracellularly before they are transported out of the cell through the basolateral membrane. Some of the unique immunological features of this disease are most probably related to the expression

profile of SLC7A7 (y^+LAT1), which is much broader than that of SLC3A1/SLC7A9 (rBAT/$b^{0,+}$AT).[29]

A mouse model, generated by inactivation of *Slc7a7*, displayed intrauterine growth retardation with only two animals surviving the neonatal period. These two surviving knockout mice were maintained on a low-protein diet and citrulline supplementation, and when introduced to a high-protein diet displayed a metabolic dysfunction almost identical to that observed in the human syndrome.[40]

HARTNUP DISORDER (OMIM #234500)

Clinical findings

First recognized in the 1950s in London as a defect of neutral amino-acid transport, Hartnup disorder became an example of how diet can unveil signs and symptoms of a multifaceted disease. The protein-restricted diet imposed to the population (World War II, postwar) revealed the defective (re)absorption of amino acids characteristic of the Hartnup disorder. Patients can present with signs and symptoms of pellagra (including light-sensitive dermatitis), intermittent cerebellar ataxia, and psychosis-like symptoms. These symptoms, generally considered the result of niacin deficiency, are thought to be caused by deficiency of tryptophan, as this is a precursor of niacin and serotonin.

Diagnostically, elevated urinary neutral amino acids are the first indication of the disorder. The intestinal malabsorption of tryptophan can be additionally tested by an indican–indole test, although this is rarely used and not readily available. The neutral aminoaciduria has to be differentiated from generalized aminoaciduria, which would be an obligate diagnostic hallmark of a renal Fanconi syndrome. The most common cause of the renal Fanconi syndrome in childhood is cystinosis, a treatable lysosomal storage disorder.

Treatment

Currently, patients from 'protein supersaturated' countries are often recognized only by newborn screening programs, as they are asymptomatic. The question remains whether these patients should or need to be treated at all. Patients with pellagra-like symptoms are treated with niacin oral replacement, which reverses the clinical features of the disorder.

Physiology and molecular genetics

The incidence of Hartnup disorder is estimated to be in the range of 1:15 000 births. Mutations in *SLC6A19*, encoding the neutral amino-acid transporter B^0AT1, mediating neutral amino-acid transport from the luminal compartment into the cells (see Figure 1), are causative for autosomal-recessive Hartnup disorder.[41,42] The molecular identification of the transporter involved came almost 50 years after the first description of the disorder by Baron and co-workers. In 1977, Hartnup disorder was hypothesized to be caused by the defect of a broad-range neutral amino-acid transport system.[43] This transport system was first characterized in brush border membrane vesicles of kidney[43,44] and jejunum,[44] where it was shown to co-transport Na^+ and various neutral amino acids.[45,46] It was not until 2004 that this transport system was molecularly identified in mice, and then in humans, as *SLC6A19* (B^0AT1).[41,42,47]

SLC6A19 (B^0AT1) co-transports Na^+ and a broad range of neutral amino acids with low affinity (millimolar range).[48,49] Unlike other members of the SLC6 family, *SLC6A19* (B^0AT1) does not transport biogenic amines or osmolites, and transport is not Cl^--dependent.[47,48] *SLC6A19* (B^0AT1) mRNA was found to be highly expressed in kidney proximal tubule and small intestine. A localization study in mice showed that *Slc6a19* (B^0AT1) is expressed in the brush border membrane of the early portion of the proximal kidney tubules (S1). In jejunum, the luminal expression of the transporter follows a gradient along villi, being low in the crypts and stronger toward the tips.[50]

Cloning of the human *SLC6A19* gene (chromosomal locus 5p15.33) led to the identification of a deleterious splice site mutation in members of the original British Hartnup family.[41] In the original two reports describing the cloning of this gene, nine further deletions, missense, nonsense, and splice site mutations causing this autosomal-recessive disorder were identified in families from Japan and Australia.[41,42]

Yet, no mouse model that mimics Hartnup disorder by disruption of the *Slc6a19* gene exists. However, a recently characterized *Tmem27* (collectrin) knockout mouse was shown to lack *Slc6A19* (B^0AT1) in the kidney and to present a massive neutral aminoaciduria without glucosuria or phosphaturia pointing to collectrin being an obligatory subunit of *Slc6A19* in the kidney.[51–53] Collectrin is predicted to be a type I transmembrane protein. Its interactions with *SLC6A19* (B^0AT1) are thought to be through non-covalent interactions. Interestingly, this gene, *TMEM27*, is located on the X chromosome. No patients with neutral amino aciduria (similar to Hartnup disorder) and X-linked recessive inheritance have been observed so far.

IMINOGLYCINURIA (OMIM %242600)

Clinical findings

Patients with iminoglycinuria present with elevated urinary levels of glycine, proline, and hydroxyproline. Interestingly, obligate heterozygotes (parents of patients) show glycinuria only.

The patients diagnosed by newborn screening programs should be subsequently confirmed, as iminoglycinuria can be a normal finding in neonates, presumably reflecting tubular immaturity. Several reports have linked iminoglycinuria to other diseases, a finding that may have been biased due to ascertainment errors resulting from the specific populations screened (for example, mental health institutions). As in other aminoacidurias, it is customary to analyze the fractional excretion of all respective amino acids to exclude urinary losses due to elevated plasma levels. Such a situation can occur in another metabolic disorder, hyperprolinemia, where urine findings can mimic iminoglycinuria

by significantly elevated plasma proline levels exceeding urinary reabsorption capacity.

Treatment

At present time, iminoglycinuria is considered an incidental finding and no treatment is necessary. Whether constant urinary glycine, proline, and hydroxyproline losses have an impact on health is not known.

Physiology and molecular genetics

The incidence of iminoglycinuria is 1:10 000 births. The molecular cause of autosomal-recessive iminoglycinuria is not known at present.

Studies with amino-acid transporters expressed in heterologous systems pointed to the possibility that mutations in *SLC36A1* or *SLC6A20* could be causative for iminoglycinuria. *SLC36A1* encodes a proton-dependent amino-acid transporter called PAT-1, and *SLC6A20* the sodium-dependent imino transporter SIT-1.

SLC36A1 (PAT-1), as presumed imino acid carrier, co-transports protons and proline, hydroxyproline, glycine, L-, D-, and β-alanine, and D-serine, with low affinity (millimolar range).[54] It is expressed significantly at mRNA level in small intestine, colon, brain, and kidney.[55] *SLC6A20* (SIT-1), as presumed IMINO transporter, co-transports Na^+, proline, methylaminoisobutyrate (MeAIB), methylproline, and hydroxyproline with high affinity (micromolar range), but not glycine.[56,57] The mRNA is highly expressed in small intestine, kidney, lungs, spleen, testis, and brain. *SLC6A20* (SIT-1) localizes to the brush border membranes of proximal tubules (S1 = S2 = S3) and enterocytes.[50]

It has been hypothesized that mutations in multiple genes, namely *SLC36A1* (PAT-1), *SLC6A20* (SIT-1), and *SLC6A19* (B^0AT1), could be the cause of iminoglycinuria.[58] However, preliminary genetic studies by us seem to exclude *SLC36A1* (PAT-1) (chromosomal locus 5q33.1) and *SLC6A20* (SIT-1) (chromosomal locus 3p21.31) as being causative for investigated cases of iminoglycinuria.[59]

DICARBOXYLIC AMINOACIDURIA (OMIM 222730)

Clinical findings

A few patients with no typical clinical presentation have been reported with an excess urinary excretion of acidic amino acids, that is, aspartate and glutamate.[8,9]

Physiology and molecular genetics

An incidence of 1:35 000 births was estimated from a 25-year screening program in Quebec (Canada).[60] At first, an inborn error in *SLC1A1* (EAAC1/EAAT3), an acidic (that is, glutamate and aspartate) amino-acid transporter (see Figure 1), was suggested to cause this disease.[61,62] The transporter is highly expressed in the later portion of the proximal tubule in kidney (S2, S3 ⩾ S1), the brush border of ileal enterocytes, and neurons of various brain areas.[63–65] *SLC1A1* (EAAC1/EAAT3) co-transports glutamate, or L- or D-aspartate with Na^+ and H^+ in exchange for K^+ from the tubule lumen into the cell with high affinity.[66] A *Slc1a1* (Eaac1/Eaat3) knockout mouse developed dicarboxylic aminoaciduria, confirming the role of EAAC1 in the reabsorption of glutamate from the renal proximal tubules.[67]

Despite all this evidence, so far no mutations in the human *SLC1A1* gene (chromosomal locus 9p24) have been identified in patients with dicarboxylic aminoaciduria.

Summary

Aminoacidurias are caused by defective amino-acid transport systems in renal and intestinal epithelia. In the last few decades, huge progress has been made in identifying genes and proteins involved in the pathology of some of these disorders. Currently, the genes involved in cystinuria, LPI, and Hartnup disorder have been identified (see Table 1, Figure 1). The study of the mutated proteins expressed in heterologous systems and of genes inactivated in mice models are tools that can be used for searching for therapies. The large screening programs of newborns can reveal different forms of aminoacidurias, symptomatic or not, and with that the identification of new transporters. Some fundamental physiological questions are not solved; for example, it is not clear why with *SLC7A7* ($b^{0,+}AT$) and *SLC7A9* (y^+LAT1) transcellular (re)absorption of dibasic amino acids is mediated by obligatory exchangers that expressed alone would produce a net secretion of neutral amino acids? What is the identity of the basolateral transporter(s) for neutral amino acids? What are the identities of the luminal and basolateral transporters for acidic amino acids? Which other proteins are responsible for the (re)absorption of imino acids and glycine? What is the basis for the immunological findings of alveolitis and glomerulonephritis in LPI? Pursuing the answers to these questions is important to help understand the complex physiology of amino-acid regulation in the body and their role in human pathophysiology. The elucidation of amino-acid transport across polarized and non-polarized cell membranes creates the possibility of modifying the natural course of diseases by interfering with these processes.

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